

### Amendments to the Specification

Please replace page 40 of the specification as follows:

Primary Screening	100 μg	Cells Lines (μg/ml)						
		HeLa	HeLa	MDA	ESG	ESG	ESG	ESG
Ph.	60	4.5E03	3.0E07	8.5E07	1.0E06	8.2E07	5.5E07	
	73	8.2E05	6.0E07	2.0E06	7.0E05	2.2E06	9.5E07	
ZK	129	1.2E05	1.2E05	1.2E05	1.2E05	8.8E05	2.0E05	

*Antimicrobial activity: On solid medium*

*Bacillus subtilis*. 10μg/disk (6mm diameter): 10 mm inhibition zone

*Spectroscopic data:*

HRFABMS *m/z* 509.275351 [M-H<sub>2</sub>O+H]<sup>+</sup> (calcd for C<sub>28</sub>H<sub>37</sub>N<sub>4</sub>O<sub>5</sub> 509.276396 Δ 1.0 mmu); LRFABMS using m-NBA as matrix *m/z* (rel intensity) 509 [M-H<sub>2</sub>O+H]<sup>+</sup> (5), 460 (2.7), 391 (3).

<sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz): 6.70 (s, H-15), 6.52 (s, H-5), 4.72 (bs, H-11), 4.66 (d, *J* = 2.0 Hz, H-21), 4.62 (dd, *J* = 8.4, 3.7 Hz, H-1), 3.98 (bd, *J* = 7.6 Hz, H-13), 3.74 (s, 7-OMe), 3.71 (s, 17-OMe), 3.63 (m, overlapped signal, H-25), 3.62 (m, overlapped signal, H-3), 3.30 (m, H-22a), 3.29 (m, H-14a), 3.18 (d, *J* = 18.6 Hz, H-14b), 2.90 (m, H-4a), 2.88 (m, H-22b), 2.76 (s, 12-NMe), 2.30 (s, 16-Me), 2.22 (m, H-4b), 1.16 (d, *J* = 7.4 Hz, H-26);

<sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz): 170.75 (s, C-24), 149.24 (s, C-18), 147.54 (s, C-8), 145.95 (s, C-7), 145.82 (s, C17), 133.93 (s, C-16), 132.31 (s, C-9), 131.30 (s, C-6), 128.95 (s, C-20), 121.93 (d, C-15), 121.76 (d, C-5), 121.44 (s, C-10), 112.45 (s, C-19), 92.87 (d, C-21), 60.86 (q, 7-OMe), 60.76 (q, 17-OMe), 59.39 (d, C-11), 57.96 (d, C-13), 55.51 (d, C-1), 54.29 (d, C-3), 50.08 (d, C-25), 45.55 (t, C-22), 40.43 (q, 12-NMe), 32.56 (t, C-4), 25.84 (t, C-14), 17.20 (q, C-26), 16.00 (q, 16-Me), 15.81 (q, 6-Me).

Cells Lines (Mol/L)																
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix		
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL	
Safra-cin P-22B	GI50	4.58E-06	3.08E-07	8.49E-07	3.02E-06	8.24E-07	5.20E-07	4.71E-06	1.13E-07	4.77E-06	1.01E-06	2.54E-06	6.95E-06	7.61E-07	4.65E-07	
	TGI	8.62E-06	6.08E-07	2.30E-06	7.04E-06	2.28E-06	9.99E-07	8.83E-06	4.67E-07	1.17E-05	2.75E-06	6.84E-06	1.90E-05	1.83E-06	9.32E-07	
	LC50	1.62E-05	1.20E-06	1.21E-05	1.65E-05	8.85E-06	2.01E-06	1.66E-05	1.84E-06	>1.90E-05	1.86E-05	1.84E-05	>1.90E-05	7.42E-06	1.86E-06	

*Antimicrobial activity:* On solid medium

*Bacillus subtilis.* 10 $\mu$ g/disk (6mm diameter): 10 mm inhibition zone

Spectroscopic data:

HRFABMS *m/z* 509.275351 [M-H<sub>2</sub>O+H]<sup>+</sup> (calcd for C<sub>28</sub>H<sub>37</sub>N<sub>4</sub>O<sub>5</sub> 509.276396 Δ 1.0 mmu);

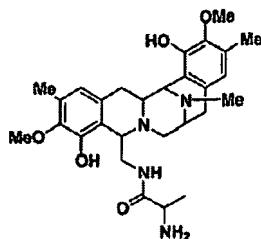
LRFABMS using m-NBA as matrix *m/z* (rel intensity) 509 [M-H<sub>2</sub>O+H]<sup>+</sup> (5), 460 (2.7), 391 (3).

<sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz): 6.70 (s, H-15), 6.52 (s, H-5), 4.72 (bs, H-11), 4.66 (d, *J* = 2.0 Hz, H-21), 4.62 (dd, *J* = 8.4, 3.7 Hz, H-1), 3.98 (bd, *J* = 7.6 Hz, H-13), 3.74 (s, 7-OMe), 3.71 (s, 17-OMe), 3.63 (m, overlapped signal, H-25), 3.62 (m, overlapped signal, H-3), 3.30 (m, H-22a), 3.29 (m, H-14a), 3.18 (d, *J* = 18.6 Hz, H-14b), 2.90 (m, H-4a), 2.88 (m, H-22b), 2.76 (s, 12-NMe), 2.30 (s, 16-Me), 2.22 (m, H-4b), 1.16 (d, *J* = 7.4 Hz, H-26);

<sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz): 170.75 (s, C-24), 149.24 (s, C-18), 147.54 (s, C-8), 145.95 (s, C-7), 145.82 (s, C17), 133.93 (s, C-16), 132.31 (s, C-9), 131.30 (s, C-6), 128.95 (s, C-20), 121.93 (d, C-15), 121.76 (d, C-5), 121.44 (s, C-10), 112.45 (s, C-19), 92.87 (d, C-21), 60.86 (q, 7-OMe), 60.76 (q, 17-OMe), 59.39 (d, C-11), 57.96 (d, C-13), 55.51 (d, C-1), 54.29 (d, C-3), 50.08 (d, C-25), 45.55 (t, C-22), 40.43 (q, 12-NMe), 32.56 (t, C-4), 25.84 (t, C-14), 17.20 (q, C-26), 16.00 (q, 16-Me), 15.81 (q, 6-Me).

Please replace page 41 of the specification as follows:

**COMPOUND P-22A**



*Strain:*

The same as for P-22B

*Fermentation conditions:*

The same as for P-22B

*Isolation:*

The same as for P-22B

*Biological activities of safracin P-22A*

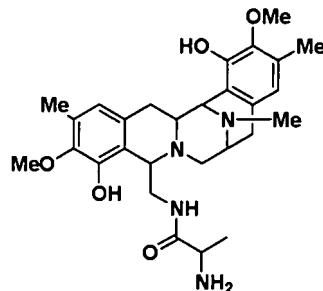
Antitumor activities

Tumor System	Cells Lines (Möller)												Oncor
	HT1080	U-157	3T3	LLC	PC3	SW1573	AGS	AGS	PC3	PC3	U-157	U-157	
Safracin P-22A	655 > 1555	4555		7255	12555	12755	13555		> 15555	15555	15555	15555	15555
	1555 > 15555	12555		13555	> 15555	> 15555	13555		> 15555	15555	> 15555	15555	15555
	1555 > 15555	15555		> 15555	15555	15555	15555		> 15555	15555	> 15555	15555	15555

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10 $\mu$ g/disk (6mm diameter): NO ACTIVE

## COMPOUND P-22A



*Strain:*

The same as for P-22B

Fermentation conditions:

The same as for P-22B

Isolation:

The same as for P-22B

### Biological activities of safracin P-22A

#### Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin P-22A	GI50	>1.96E-05	4.19E-06	7.74E-06	1.30E-05	1.27E-05	5.93E-06	>1.96E-05	3.15E-06	>1.96E-05	1.26E-05	>1.96E-05	>1.96E-05	8.75E-06	7.66E-06
	TD50	>1.96E-05	9.26E-06	1.96E-05	>1.96E-05	>1.96E-05	1.33E-05	>1.96E-05	7.93E-06	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05
	LC50	>1.96E-05	1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05						

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10 $\mu$ g/disk (6mm diameter): NO ACTIVE

Please replace page 43 of the specification as follows:

*Biological activities of safracin P-19B*

Antitumor activities

Tumor bearing animal	Product	Cells Lines (Möller)													
		3T3	9L	KR-2	LOX	LEW	PC-3	PC-3M	PC-3M2	PC-3M2-A	PC-3M2-B	PC-3M2-C	PC-3M2-D	PC-3M2-E	PC-3M2-F
3T3-MCF	660	100E6	100E6		5.0E5	8.0E5	7.0E5	7.0E5		>1.0E5	2.0E5	1.0E5	1.0E5	>1.0E5	1.0E5
3T3	660	>1.0E5	1.0E5		1.0E5	>1.0E5	1.0E5	1.0E5	>1.0E5	>1.0E5	1.0E5	>1.0E5	1.0E5	1.0E5	1.0E5
2407Q2	100	>1.0E5	1.0E5		>1.0E5	>1.0E5	>1.0E5	>1.0E5	>1.0E5	>1.0E5	1.0E5	>1.0E5	1.0E5	>1.0E5	1.0E5

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6mm diameter): NO ACTIVE

*Spectroscopic data:*

HRFABMS *m/z* 495.260410 [M-H<sub>2</sub>O+H]<sup>+</sup> (calcd for C<sub>27</sub>H<sub>35</sub>N<sub>4</sub>O<sub>5</sub> 495.260746 Δ 0.3 mmu); LRFABMS using m-NBA as matrix *m/z* (rel intensity) 495 [M-H<sub>2</sub>O+H]<sup>+</sup> (13), 460 (3), 391 (2); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz): 6.67 (s, H-15), 6.5 (s, H-5), 3.73 (s, 7-OMe), 3.71 (s, 17-OMe), 2.29 (s, 16-Me), 2.24 (s, 6-Me), 1.13 (d, *J* = 7.1 Hz, H-26);

## Biological activities of safracin P-19B

Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVODOX	HELA	HELA-APL
Safracin P-19B	GI50	1.70E-05	3.90E-06	5.42E-06	8.74E-06	7.08E-06	7.90E-06	>1.95E-05	2.38E-06	1.81E-05	1.55E-05	>1.95E-05	1.44E-05	6.73E-06	4.80E-06
	TGI	>1.95E-05	8.06E-06	1.48E-05	>1.95E-05	1.92E-05	>1.95E-05	>1.95E-05	5.77E-06	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.61E-05	1.00E-05
	LC50	>1.95E-05	1.67E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.40E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.95E-05

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6mm diameter): NO ACTIVE

Spectroscopic data:

HRFABMS *m/z* 495.260410 [M-H<sub>2</sub>O+H]<sup>+</sup> (calcd for C<sub>27</sub>H<sub>35</sub>N<sub>4</sub>O<sub>5</sub> 495.260746 Δ 0.3 mmu);

LRFABMS using m-NBA as matrix *m/z* (rel intensity) 495 [M-H<sub>2</sub>O+H]<sup>+</sup> (13), 460 (3), 391 (2);

<sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz): 6.67 (s, H-15), 6.5 (s, H-5), 3.73 (s, 7-OMe), 3.71 (s, 17-OMe), 2.29 (s, 16-Me), 2.24 (s, 6-Me), 1.13 (d, *J* = 7.1 Hz, H-26);

Please replace page 46 of the specification as follows:

Cells Lines (Mol/L)																	
Primary Screening	Proteins		Oncop		Breed		Mammal		Fowl		HCV		Lymphoma				
	10 <sup>-10</sup>	10 <sup>-9</sup>	10 <sup>-8</sup>	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	10 <sup>-3</sup>	10 <sup>-2</sup>	10 <sup>-1</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	10 <sup>-3</sup>			
PN - Fernando de la Calle (2)	100	5.22E-03	1.54E-03		2.59E-03	1.30E-03	4.71E-03	2.51E-03		6.01E-03	6.01E-03	4.71E-03	4.33E-03	1.30E-03	4.23E-03	2.25E-03	1.22E-03
T01	100	3.03E-03	4.12E-03		6.02E-03	3.31E-03	7.02E-03	6.21E-03		1.07E-03	1.05E-03	1.05E-03	1.73E-03	1.07E-03	6.95E-03	6.95E-03	5.20E-03
94U502	100	1.93E-03	3.71E-03		1.35E-03	9.45E-03	1.92E-03	1.58E-03		1.11E-03	>1.05E-03	>1.05E-03	>1.05E-03	>1.05E-03	1.65E-03	1.32E-03	1.21E-03

Secondary Evaluation (Mol/L)														
Secondary Screening	Anticancer		Antivirals		Antibiotics		Other		Oncop		Antibiotics		Other	
	10 <sup>-10</sup>	10 <sup>-9</sup>	10 <sup>-8</sup>	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	10 <sup>-3</sup>	10 <sup>-2</sup>	10 <sup>-1</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	10 <sup>-3</sup>
PN - Fernando de la Calle 20-43602	100	1.95E-03	1.52E-03	3.20E-03	2.85E-03	6.05E-03								

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10 $\mu$ g/disk (6mm diameter): Inhibition zone: 15 mm diameter

Spectroscopic data

ESMS:  $m/z$  509 [M-H<sub>2</sub>O+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.50 (s, C-15), 4.02 (s, OMe), 3.73 (s, OMe), 2.22 (s, Me), 1.85 (s, Me), 0.80 (d,  $J$  = 7.2 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): 186.51, 181.15, 175.83, 156.59, 145.09, 142.59, 140.78, 137.84, 131.20, 129.01, 126.88, 121.57 (2 x C), 82.59, 60.92, 60.69, 53.12, 21.40, 50.68, 50.22, 48.68, 40.57, 29.60, 25.01, 21.46, 15.64, 8.44.

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin D	GI50	5.22E-06	1.54E-06	2.68E-06	1.33E-06	4.71E-06	3.51E-06	6.04E-06	6.04E-07	4.77E-06	4.33E-06	6.99E-06	4.75E-06	3.76E-06	2.28E-06
	TGI	9.99E-06	4.12E-06	6.02E-06	3.34E-06	7.82E-06	6.21E-06	1.07E-05	1.16E-06	1.10E-05	1.79E-05	1.82E-05	8.85E-06	6.68E-06	5.24E-06
	LC50	1.90E-05	9.78E-06	1.35E-05	9.15E-06	1.30E-05	1.10E-05	1.88E-05	3.78E-06	>1.90E-05	>1.90E-05	>1.90E-05	1.65E-05	1.19E-05	1.21E-05

Secondary Evaluation (Mol/L)														
Secondary Screening				Macromolecules Synthesis					Apoptosis		DNA Binding			
				PROTEIN		DNA		RNA	NUCLEOSOMES		GEL			
Safracin D		IC50		1.90E-05		1.52E-05		3.80E-06	2.85E-06		6.65E-06			

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10 $\mu$ g/disk (6mm diameter): Inhibition zone: 15 mm diameter

#### Spectroscopic data

ESMS:  $m/z$  509 [M-H<sub>2</sub>O+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.50 (s, C-15), 4.02 (s, OMe), 3.73 (s, OMe), 2.22 (s, Me), 1.85 (s, Me), 0.80 (d,  $J$  = 7.2 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): 186.51, 181.15, 175.83, 156.59, 145.09, 142.59, 140.78, 137.84, 131.20, 129.01, 126.88, 121.57 (2 x C), 82.59, 60.92, 60.69, 53.12, 21.40, 50.68, 50.22, 48.68, 40.57, 29.60, 25.01, 21.46, 15.64, 8.44.

Please replace page 48 of the specification as follows:

Primary Screening	Cells Lines (Mö/L)																	
	3T3	HeLa	MDA-MB-231	MDA-MB-436	MDA-MB-468	MDA-MB-468T	MDA-MB-468T2	MDA-MB-468T3	MDA-MB-468T4	MDA-MB-468T5	MDA-MB-468T6	MDA-MB-468T7						
PU - Fernando de la Coba 02	620	9.31E-01	1.02E-06		1.57E-05	1.64E-03	1.02E-06	1.97E-03		1.65E-03	4.23E-03	1.61E-03	7.09E-03	7.13E-03	4.07E-03	4.18E-03	1.02E-0	
1940042	160	1.93E-05	7.20E-05		1.05E-05	1.25E-03	1.24E-05	9.35E-03		>1.92E-05	6.21E-03	1.47E-03	1.93E-05	>1.92E-05	1.93E-05	1.93E-05	7.28E-03	7.25E-0

Secondary Evaluation (Mö/L)													
Secondary Screening	Microorganisms			Fungi			Plants			Insects			
	1000	100	10	1000	100	10	1000	100	10	1000	100	10	
PU - Fernando de la Coba 02	100				1.57E-03		>1.92E-05						

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6mm diameter): 9.5 mm inhibition zone

Spectroscopic data

ESMS: *m/z* 511 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.51 (s, C-15), 4.04 (s, OMe), 3.75 (s, OMe), 2.23 (s, Me), 1.89 (s, Me), 0.84 (d, *J* = 6.6 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): 186.32, 181.28, 175.83, 156.43, 145.27, 142.75, 141.05, 137.00, 132.63, 128.67, 126.64, 122.00, 120.69, 60.69, 60.21, 59.12, 58.04, 57.89, 50.12, 49.20, 46.72, 39.88, 32.22, 25.33, 21.29, 15.44, 8.23.

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANCI	HT29	LOVO	LOVODOX	HELA	HELA-APL
Safracin E	GI50	8.34E-06	3.86E-06	4.50E-06	4.54E-06	5.05E-06	3.94E-06	1.96E-05	4.25E-06	6.05E-06	7.89E-06	7.15E-06	5.07E-06	4.15E-06	4.03E-06
	TGI	1.96E-05	7.70E-06	8.85E-06	8.25E-06	9.24E-06	6.93E-06	>1.96E-05	8.21E-06	1.47E-05	1.96E-05	>1.96E-05	9.44E-06	7.29E-06	7.25E-06
	LC50	>1.96E-05	1.54E-05	1.74E-05	1.49E-05	1.70E-05	1.22E-05	>1.96E-05	1.59E-05	>1.96E-05	>1.96E-05	>1.96E-05	1.75E-05	1.28E-05	1.30E-05

Secondary Evaluation (Mol/L)														
Secondary Screening				Macromolecules Synthesis				Apoptosis			DNA Binding			
				PROTEIN	DNA	RNA	NUCLEOSOMES	GEL						
Safracin E	IC50						1.57E-05			>1.96E-05				

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6mm diameter): 9.5 mm inhibition zone

Spectroscopic data

ESMS: *m/z* 511 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.51 (s, C-15), 4.04 (s, OMe), 3.75 (s, OMe), 2.23 (s, Me), 1.89 (s, Me), 0.84 (d, *J* = 6.6 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): 186.32, 181.28, 175.83, 156.43, 145.27, 142.75, 141.05, 137.00, 132.63, 128.67, 126.64, 122.00, 120.69, 60.69, 60.21, 59.12, 58.04, 57.89, 50.12, 49.20, 46.72, 39.88, 32.22, 25.33, 21.29, 15.44, 8.23.

Please replace page 52 of the specification as follows:

cells. The clarified broth (765 ml) was adjusted to pH 9.0 by NaOH 10%. Then, the alkali-clarified broth was extracted with 1:1 (v/v) EtOAc (x2). The organic phase was evaporated under high vacuum and a greasy-dark extract was obtained (302 mg).

This extract was washed by an hexane trituration for removing impurities and the solids were purified by a chromatography column using Silica normal-phase and a mixture of Ethyl Acetate: Methanol (from 12:1 to 1:1). The fractions were analyzed under UV on TLC (Silica 60, mobile phase EtOAc:MeOH 5:4. Rf 0.3 (Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

*Biological activities of safracin B (OEt)*

Antitumor activities

Cells Lines (MöL)																
Primary Screening	Prostate		Ovary		Breast		Melanoma		Lung		NSCLC		Leukemia		Pancreas	
	PC-3	LNCaP	IGROV1	SKOV3	MDA-MB-231	MDA-MB-436	HT-29	LOVO	SW620	SW480	NCI-H460	NCI-H358	NCI-H3127	NCI-H3580	NCI-H596	
Screen 1	0.05	LM1507	LM1508		LM1509	LM1507	LM1508	LM1509		LM1507	LM1508	LM1507	LM1508	LM1507	LM1508	
Screen 2	1G	LM1506	> LM1505		LM1503	LM1507	LM1509	LM1508		LM1503	LM1507	LM1506	LM1505	LM1507	LM1508	
Z-0042	1G	LM1504	LM1507		LM1505	LM1506	LM1508	LM1509		LM1505	LM1503	LM1506	> LM1505	> LM1505	LM1505	

Secondary Evaluation (MöL)																
Secondary Screening	Prostate		Ovary		Breast		Melanoma		Lung		NSCLC		Leukemia		Pancreas	
	PC-3	LNCaP	IGROV1	SKOV3	MDA-MB-231	MDA-MB-436	HT-29	LOVO	SW620	SW480	NCI-H460	NCI-H358	NCI-H3127	NCI-H3580	NCI-H596	
	10000	1000	> 1000	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000	

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6 mm diameter): 17,5 mm inhibition zone

Spectroscopic data:

ESMS: m/z 551 [M-H<sub>2</sub>O+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.48 (s, H-15), 2.31 (s, 16-Me), 2.22 (s, 12-NMe), 1.88 (s, 6-Me), 1.43 (t, J = 6.9 Hz, Me-Etoxy), 1.35 (t, J = 6.9 Hz, Me-Etoxy), 0.81 (d, J = 7.2 Hz, H-26)

cells. The clarified broth (765 ml) was adjusted to pH 9.0 by NaOH 10%. Then, the alkali-clarified broth was extracted with 1:1 (v/v) EtOAc (x2). The organic phase was evaporated under high vacuum and a greasy-dark extract was obtained (302 mg).

This extract was washed by an hexane trituration for removing impurities and the solids were purified by a chromatography column using Silica normal-phase and a mixture of Ethyl Acetate: Methanol (from 12:1 to 1:1). The fractions were analyzed under UV on TLC (Silica 60, mobile phase EtOAc:MeOH 5:4. Rf 0.3 (Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

#### Biological activities of safracin B (OEt)

##### Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin B (OEt)	GI50	4.01E-07	4.84E-08	4.06E-08	6.82E-07	4.82E-08	1.69E-07	5.01E-07	3.97E-08	6.49E-07	2.44E-07	4.43E-07	2.09E-06	8.92E-08	7.70E-08
	TGI	1.01E-06	>1.76E-05	9.97E-08	1.19E-06	1.16E-07	4.40E-07	1.16E-06	1.08E-07	2.06E-06	1.39E-06	1.09E-06	9.88E-06	3.15E-07	2.74E-07
	LC50	1.60E-05	8.28E-07	4.27E-06	6.37E-06	1.02E-06	1.13E-06	5.66E-06	3.69E-06	1.35E-05	>1.76E-05	>1.76E-05	>1.76E-05	1.35E-06	9.76E-07

Secondary Evaluation (Mol/L)														
Secondary Screening			Macromolecules Synthesis					Apoptosis			DNA Binding			
			PROTEIN		DNA	RNA	NUCLEOSOMES		GEL					
Safracin B (OEt)		IC50	>1.76E-05			1.76E-06	1.76E-07			5.28E-08			1.76E-05	

##### Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6 mm diameter): 17,5 mm inhibition zone

##### Spectroscopic data:

ESMS: m/z 551 [M-H<sub>2</sub>O+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.48 (s, H-15), 2.31 (s, 16-Me), 2.22 (s, 12-NMe), 1.88 (s, 6-Me), 1.43 (t, J = 6.9 Hz, Me-Etoxy), 1.35 (t, J = 6.9 Hz, Me-Etoxy), 0.81 (d, J = 7.2 Hz, H-26)

Please replace page 54 of the specification as follows:

[REDACTED]

EtOAc:MeOH 5:4. Rf 0.3 Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

*Biological activities of safracin A (OEt):*

Antitumor activities

Cells Lines (Mol/L)														
Primary Screening	J774		L929		3T3		Oxy		Molt		V79		HepG2	
	IC50	ED50	IC50	ED50	IC50	ED50	IC50	ED50	IC50	ED50	IC50	ED50	IC50	ED50
Safracin A-OEt [1]	650	2.6E-06	3.2E-07		4.2E-07	2.9E-08	6.8E-07	7.9E-07		4.0E-08	8.1E-07	3.0E-06	1.9E-03	2.0E-05
T98	5.2E-08	7.1E-03		6.2E-07	5.1E-08	1.2E-08	1.9E-08		7.1E-08	8.0E-07	6.3E-06	4.4E-06	4.4E-06	2.9E-08
2A3102	1.09	1.05E-05	1.4E-06		1.6E-05	1.0E-06	5.7E-05	4.7E-05		1.3E-05	1.5E-05	1.1E-05	3.8E-05	9.1E-05
													1.9E-05	1.8E-05

Secondary Evaluation (Mol/L)														
Secondary Screening	L929		A549		Molt		V79		HepG2		PC3		L1210	
	IC50	ED50	IC50	ED50	IC50	ED50	IC50	ED50	IC50	ED50	IC50	ED50	IC50	ED50
	1.04E-06	ED			6.3E-06	1.9E-05								

Antimicrobial activity: On solid medium

*Bacillus subtilis.* 10 $\mu$ g/disk (6 mm diameter): 10 mm inhibition zone

Spectroscopic data:

ESMS: m/z 553 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.48 (s, H-15), 2.33 (s, 16-Me), 2.21 (s, 12-NMe), 1.88 (s, 6-Me), 1.42 (t, J = 6.9 Hz, Me-Etoxy), 1.34 (t, J = 6.9 Hz, Me-Etoxy), 0.8 (d, J = 6.9 Hz, H-26)

EtOAc:MeOH 5:4. Rf 0.3 Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

Biological activities of safracin A (OEt):

Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin A (OEt)	GI50	2.64E-06	3.78E-07	4.92E-07	2.01E-06	5.55E-07	7.96E-07	4.00E-06	3.11E-07	3.06E-06	1.97E-06	2.03E-06	5.72E-06	1.02E-06	7.64E-07
	TD50	5.39E-06	7.42E-07	9.28E-07	5.10E-06	1.16E-06	1.90E-06	7.17E-06	6.86E-07	5.83E-06	4.41E-06	4.41E-06	9.84E-06	2.91E-06	2.32E-06
	LC50	1.10E-05	1.45E-06	1.76E-06	1.30E-05	5.57E-06	5.77E-06	1.28E-05	1.51E-06	1.11E-05	9.88E-06	9.88E-06	1.69E-05	7.85E-06	6.69E-06

Secondary Evaluation (Mol/L)									
Secondary Screening			Macromolecules Synthesis				Apoptosis		DNA Binding
			PROTEIN	DNA	RNA	NUCLEOSOMES	GEL		
Safracin A (OEt)	IC50				6.33E-06	1.81E-06			

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10 $\mu$ g/disk (6 mm diameter): 10 mm inhibition zone

Spectroscopic data:

ESMS: m/z 553 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.48 (s, H-15), 2.33 (s, 16-Me), 2.21 (s, 12-NMe), 1.88 (s, 6-Me), 1.42 (t, J = 6.9 Hz, Me-Etoxy), 1.34 (t, J = 6.9 Hz, Me-Etoxy), 0.8 (d, J = 6.9 Hz, H-26)